myocytes, where the native Ito is small, simulation of an epicardial-level Ito accentuated the phase 1 repolarization and significantly suppressed cell shortening by 19%. The peak amplitude of Ca2+ transient was also reduced in the presence of simulated Ito, although the rate of rise of the Ca2+ transient was increased. Conversely, subtraction, or "blockade" of the native Ito using the dynamic clamp enhanced contractility in epicardial cells. These results agree with the inverse correlation between Ito levels and myocyte contractility and Ca2+ transient amplitude in epicardial and endocardial myocytes. Action potential clamp and computational modeling show that phase-1 notch depth vs peak L-type influx has an inverted-U shape; shallow phase-1 notch enhances Ica-L peak, while moderate to strong phase-1 repolarization reduces Ica-L influx.
Conclusion: Our results show that Ito acts as a negative, rather than positive regulator of myocyte mechanical properties in large animals.

1865-Pos
FRET Microscopy Reveals that Phospholamban Binds More Avidly to SERCA1a than SERCA2a
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SERCA1a and SERCA2a have been used interchangeably in mutagenic and structural analyses of the PLB-SERCA interaction, since in vitro studies have shown their functional inhibition by PLB is equivalent. To quantify the quaternary structure and binding energetics of PLB binding to SERCA isoforms, fluorescence resonance energy transfer (FRET) from Cer-SERCA1a or Cer-SERCA2a to YFP-PLB was measured in live AAV-293 cells. FRET efficiency increased with increasing protein expression level to a maximum of 28.8% for PLB-SERCA1a and 28.1% for PLB-SERCA2a, suggesting the complexes have the same quaternary conformation. Unexpectedly, the data also revealed that PLB has a 2.6 fold higher apparent affinity for SERCA1a relative to SERCA2a. To test whether the observed difference in affinity arises from differential distributions of SERCA E1/E2 enzymatic substates, cells were treated with 1mM EGTA and 0.5uL/mL calcium ionophore A23187. Under these conditions, PLB still showed greater affinity for SERCA1a over SERCA2a, suggesting that the differential affinities are intrinsic properties of the SERCA isoforms. The data suggest that PLB preferentially binds SERCA1a over SERCA2a, which may be an important strategic consideration for therapeutic overexpression of SERCA isoforms in cardiac muscle.

1866-Pos
Dislocations and Helicoids in Myofibrillar Z-Disks of Mammalian Ventricular Myocytes and Implications for Calcium Handling
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The sarcomeric organization in rat ventricular myocytes has been examined using confocal microscopy to clarify the detailed 3D structure of myofibrillar z-disks. Dislocations across z-disks visualized by immuno-labeling of α-actinin were present in myocytes at slack (~1.8 μm) and long sarcomere lengths (~2.2 μm). The dislocations coincided with variations in myofibrillar direction and often myofibrils appeared to be twisted along the cell length. 3-D visualization and segmentation at high resolution revealed that z-disks in these regions often were in a helicoid arrangement that extended over ~15-20 sarcomeres. Similar z-disk topology was also observed in rabbit and human ventricular cells suggesting a common role in maintaining cell structure and sarcomere assembly. Dual color fluorescence imaging of α-actinin and ryanodine receptor (RyR) clusters demonstrated that their placement at z-lines also resulted in helicoid arrangements in regions of z-disk dislocations. As a result the effective axial spacing between RyR clusters was smaller than the sarcomere length. Rat, rabbit and human t-systems in areas of dislocations were studied by labeling with anti-caveolin-3 and wheat germ agglutinin. Most t-tubules closely followed the z-lines in these species, although a perfect helicoid architecture was not observed due to t-system elements that extended in axial or oblique directions.To investigate the consequences of the complex arrangement of z-lines a model of stochastic Ca^{2+} dynamics was constructed based on the distribution of Ca^{2+} release units (CRUs) that were experimentally determined from high resolution confocal RyR data. This demonstrated the importance of the non-planar CRU arrangement in sustaining Ca^{2+} waves that spread axially in conditions of simulated overload. We conclude that the complex organization of z-disks and CRUs must be captured in detailed mechanistic models.

1867-Pos
Caveolae Differentially Control Phosphorylation of Sarcoplasmic Reticular Proteins Following β_2 Adrenoceptor Stimulation in the Adult Cardiac Myocyte
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Caveolae, small flask-like lipid rafts, play a key role in shaping the spatial characteristics of the β_2-adrenoceptor cAMP signal and confining this to the sarcolemmal compartment in the adult cardiac myocyte. Here we determine the consequences of disrupting caveolae for the ability of β_2 signalling to target sarcoplasmic reticular proteins phospholamban (PLB) and the ryanodine receptor (RyR). Experiments were performed with dissociated adult rat ventricular myocytes. Selective β_2 adrenoceptor stimulation was achieved with 10 μM zinterol in the presence of 300 nM CGP20712A (CGP). Disruption of caveolae (using the cholesterol depleting agent methyl-β-cyclodextrin, MBCD) resulted in inotropic and lusitropic responses to β_2 stimulation (70.2 ± 9.7% increase in shortening; 13.3 ± 1.3 % decrease in time to half relaxation) which were absent in control cells (n=12-20 myocytes, $P<0.001$). PLB contributes to inotropic and lusitropic responses via protein kinase A (PKA)-dependent phosphorylation at Ser^{16}. In agreement with functional data, MBCD-treated myocytes showed a marked 561 ± 144% increase in Ser^{16}-phosphorylated PLB in response to β_2 stimulation (relative to that in cells exposed to CGP alone) which was absent in control cells (93 ± 31% of that with CGP alone) (n=4, $P<0.05$). By contrast, we saw no significant increase ($P>0.05$) in phosphorylation of one of the PKA-targeted sites of RyR, Ser^{2809}, in either control (112 ± 11%) or MBCD-treated (116 ± 18%) myocytes in response to β_2 stimulation (n=5). These preliminary data suggest that caveolae selectively control cAMP signals even within the same broad (sarcoplasmic reticular) compartment of the adult cardiac myocyte. Disruption of caveolae allows β_2 cAMP-dependent signalling to access a sub-compartment of the sarcoplasmic reticulum which contains PLB, but not one which contains RyR.

1868-Pos
Solute Transport in the Transverse Tubules of Cardiac Ventricular Myocytes
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Electrical excitation in mammalian cardiac ventricular myocytes underlies the activation of cell-wide Ca^{2+} release and hence contraction. This process can occur rapidly in relatively large myocytes because transverse tubules (TTs) penetrate deep into the cells. The TT network also permits extracellular solute to be carried into the cell volume and thereby allows for improved inflow and egress of substrates. We have studied TT morphology in relaxed and contracted cells with the aim to characterize transport function and physical properties of the TT system.
Using living isolated rat ventricular myocytes, we examined the movement of substrate within the TTs under different conditions using sulforhodamine B and fluorescence imaging. In addition, we examined the TT network with respect to its size, shape and complexity using super-resolution STED (stimulated emission depletion) microscopy. The lipophilic indicator Di-8-ANEPPS was used to identify and characterize the TTs. A rapid bulk solution changer was used to measure the extracellular marker sulforhodamine B concentration changes within the T-tubule matrix at rest and during field stimulation. STED imaging of resting myocytes revealed tubules of about 250 nm in diameter (see related abstract: Wagner et al. 2010). We hypothesized that if TTs collapse and expand dynamically during contraction, solute within the TT network would exchange more rapidly during contractions than when the myocytes are quiescent. Testing this hypothesis by rapid solution change revealed that TT solute exchange was significantly faster during stimulated contraction in single heart cells. Consistent with this finding was the observation that inhibition of contraction by cytochalasin D treatment in paced myocytes reduced the rate of solute exchange. These results suggest that TT solute exchange is accelerated by the mechanical deformation of the TTs during contraction. Future work should enable us to test this hypothesis more directly.